1 **Chimeric anti-HLA antibody receptor engineered human regulatory T cells suppress**

2 **alloantigen-specific B cells from pre-sensitized transplant recipients**

3

4 Jaime Valentín-Quiroga[1,2,3], Alejandro Zarauza-Santoveña[4], Eduardo López-Collazo[3], Leonardo

5 M.R. Ferreira[1,2,*]

6

7 [1]Department of Pharmacology and Immunology, Medical University of South Carolina,

8 Charleston, SC, USA

9

10 [2]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA

11

12 [3]The Innate Immune Response Group, Hospital La Paz Institute for Health Research (IdiPAZ),

13 University Hospital La Paz, Madrid, Spain

14

15 [4]Paediatric Nephrology Unit, University Hospital La Paz, Madrid, Spain

16

17 *Corresponding author: ferreirl@musc.edu

18

19 **Abstract**

20 Organ transplantation is a lifesaving procedure, with 50,000 transplants happening every year in

21 the United States. However, many patients harbor antibodies and B cells directed against

22 allogeneic human leukocyte antigen (HLA) molecules, notably HLA-A2, greatly decreasing their

23 likelihood of receiving a compatible organ. Moreover, antibody-mediated rejection is a significant

24 contributor to chronic transplant rejection. Current strategies to desensitize patients non-

25 specifically target circulating antibodies and B cells, resulting in poor efficacy and complications.

26 Regulatory T cells (Tregs) are immune cells dedicated to suppressing specific immune responses

27 by interacting with both innate and adaptive immune cells. Here, we genetically modified human

28 Tregs with a chimeric anti-HLA antibody receptor (CHAR) consisting of an extracellular HLA-A2

29 protein fused to a CD28-CD3zeta intracellular signaling domain, driving Treg activation upon

30 recognition of anti-HLA-A2 antibodies on the surface of alloreactive B cells. We find that HLA-A2

31 CHAR Tregs get activated specifically by anti-HLA-A2 antibody-producing cells. Of note, HLA-A2

32 CHAR activation does not negatively affect Treg stability, as measured by expression of the Treg

33 lineage transcription factors FOXP3 and HELIOS. Interestingly, HLA-A2 CHAR Tregs are not

34 cytotoxic towards anti-HLA-A2 antibody-producing cells, unlike HLA-A2 CHAR modified

35 conventional CD4$^+$ T cells. Importantly, HLA-A2 CHAR Tregs recognize and significantly suppress

36 high affinity IgG antibody production by B cells from HLA-A2 sensitized patients. Altogether, our

37 results provide proof-of-concept of a new strategy to specifically inhibit alloreactive B cells to

38 desensitize transplant recipients.

39

40 **Keywords:** HLA sensitization, regulatory T cells, B cells, antibody production, transplantation,

41 engineered immune receptors, human immunology

42

43 **Introduction**

44 Organ transplantation represents a pivotal advancement in modern medicine, offering a lifeline to

45 thousands of patients suffering from end-stage organ failure. Renal transplant is the most

46 common organ transplant worldwide, according to the latest Global Observatory on Donation and

47 Transplantation (GDOT) report [1; 2].

48 However, several hurdles remain in the field of organ transplantation. A significant barrier to

49 successful transplantation is immune rejection of the donor organ by the recipient's immune

50 system [3]. The current standard of care in organ transplant patients involves lifetime multimodal

51 immunosuppressive drug therapy. Broad suppression of the immune suppression by these drugs

52   results in multiple toxicities, including viral infections, nephrotoxicity, neurotoxicity, hyperglycemia,

53   and cancer development [4; 5; 6; 7; 8]. These side effects are especially pernicious in pediatric

54   transplant recipients, who can suffer growth delays, cognitive impairments, and compounded

55   cancer risk due to continued exposure to steroids and neurotoxic drugs during a critical

56   developmental period [9; 10; 11; 12].

57   Strikingly, 20% of first-time organ recipients and up to 75% of second-time recipients harbor

58   antibodies and B cells directed against allogeneic human leukocyte antigen (HLA) molecules [13;

59   14; 15]. HLA-A2 is a very common HLA allele group, with reports of 25% of renal transplant

60   recipients in Europe and the United States receiving an HLA-A2 mismatched renal transplant [16;

61   17]. These HLA sensitized patients face an uphill battle in securing compatible grafts as the risk

62   of antibody-mediated rejection escalates, narrowing the pool of eligible donor organs [18],

63   creating the need for higher doses of immunosuppressants [19], and contributing to chronic

64   rejection [20].

65

66   Current desensitization protocols to mitigate the effects of these antibodies lack specificity,

67   targeting total circulating antibodies or B cells, resulting in poor efficacy and unintended

68   complications [19]. It is thus imperative to develop treatments that specifically target the recipient's

69   alloreactive B cells. Using a cellular approach instead of a broad pharmacological approach could

70   increase efficacy and help prevent non-specific side effects.

71

72   Regulatory T cells (Tregs), a subset of CD4$^+$ T cells integral to the maintenance of immune

73   tolerance, play a pivotal role in modulating immune responses against self and non-self antigens.

74   These specialized immune cells exert their suppressive functions through various mechanisms,

75   including the inhibition of effector T cell activation and the modulation of B cell responses [21; 22;

76   23]. Due to these tolerogenic properties, several ongoing trials focus on using Tregs as cellular

77   therapeutics to replace or diminish the dose of immunosuppressive drugs needed to prevent

78   organ transplant rejection [22; 24; 25; 26].

79

80   One strategy to reverse HLA pre-sensitization is thus to engineer Tregs to recognize anti-

81   allogeneic HLA B cells and suppress their function, leading to immune tolerance specifically to

82   the target allogeneic HLA molecule without affecting immunity to other antigens. Such an

83   approach can help more patients become eligible for organ transplants and reduce the need for

84   broad immunosuppressive regimens in transplant recipients. To accomplish this, we genetically

85   modified human Tregs with a chimeric anti-HLA antibody receptor (CHAR) consisting of an

86   extracellular HLA-A2 protein fused to a CD28-CD3zeta intracellular signaling domain [27; 28],

87   driving Treg activation upon recognition of anti-HLA-A2 antibodies on the surface of alloreactive

88   B cells, and assessed HLA-A2 CHAR Treg phenotype and suppressive function towards HLA-A2

89   sensitized patient B cells.

90

91   **Materials and Methods**

92

93   ***Molecular Biology***

94   The HLA-A2-CHAR-2A-NGRFt lentiviral plasmid was synthesized by VectorBuilder (Chicago, IL).

95   The construct contained an MND promoter driving the expression of HLA-A2 fused to a CD8 hinge

96   (H), CD28 transmembrane domain (TM), and a CD28−CD3zeta tandem intracellular signaling

97   domain (Figure 1A), followed by a T2A sequence and a truncated nerve growth factor receptor

98   (NGFRt) as a reporter gene, similar to constructs reported in [27; 28]. Lentivirus particles were

99   produced by VectorBuilder and shipped to the laboratory, where they were stored in aliquots at -

100   80°C until use.

101

102   ***Treg sorting, transduction, and expansion***

103   Human Treg isolation, lentiviral transduction, and ex vivo expansion was carried out as previously

104   described [29]. Human peripheral blood leukopaks from de-identified HLA-A2 negative healthy

105   donors were purchased from STEMCELL Technologies (Vancouver, Canada). CD4$^+$ T cells were

106   enriched using the EasySep Human CD4+ T Cell Isolation Kit (STEMCELL Technologies),

107   following manufacturer's instructions. Enriched CD4$^+$ T cells were then stained for CD4, CD25,

108   and CD127, and CD4$^+$CD25^{hi}CD127^{low} regulatory T cells (Tregs), previously shown to be *bona*

109   *fide* Tregs [30; 31], and CD4$^+$CD25^{low}CD127^{hi} effector T (Teff) cells were purified by fluorescence-

110   assisted cell sorting (FACS) using a BD FACS Aria II Cell Sorter (Beckton Dickinson, Franklin

111   Lakes, NJ). Post-sort analyses confirmed greater than 99% purity. Tregs were activated in

112   complete medium (RPMI10), comprising RPMI 1640 medium supplemented with 10% fetal bovine

113   serum (FBS), glutamax, penicillin-streptomycin, HEPES, non-essential amino acids (NEAA), and

114   sodium pyruvate (all from Gibco, ThermoFisher Scientific) with anti-CD3/CD28 beads (Gibco,

115   ThermoFisher Scientific) at a 1:1 ratio and 1,000 international units (IU) per ml of recombinant

116   human IL-2 (Peprotech, ThermoFisher Scientific) at 10^6 cells/ml in 24-wells [32]. 48 hours after

117   activation, Tregs were transduced with CHAR lentivirus at a multiplicity of infection (MOI) of 3 (3

118   particles per cell) in the presence of IL-2. After adding the lentivirus, Tregs cells were centrifuged

119   at 1,000 g at 32°C for 1 hour. Following transduction, Tregs were maintained and expanded in

120   RPMI10, with fresh medium and IL-2 being given every two days. Tregs received 1,000 IU/ml IL-

121   2 and CD4$^+$ Teff cells received 100 IU/ml IL-2 for 9-12 days. CHAR transduction efficiency was

122   evaluated by flow cytometry based on HLA2 and NGFRt reporter surface expression.

123

124   ***Activation assay***

125   CHAR Tregs were co-cultured with irradiated (4,000 rad) B-cell hybridoma cell lines (kind gift from

126   Instituto Salud Carlos III) specific for HLA-A2 (PA2.1) or HLA-DR (IVA12) at a 1:1 ratio in RPMI10

127   medium supplemented with 1,000 IU/ml IL-2 in round-bottom 96-wells. Tregs alone served as a

128   negative control. Surface expression of the T cell activation marker CD69 in CHAR$^+$ Tregs was

129   assessed 48 hours later by flow cytometry. Parallel co-cultures were kept for 9 days to assess

130   the frequency of CHAR$^+$ Tregs, as measured by surface expression of NGFR, as an additional

131   metric of activation.

132

133   ***Treg stability assessment***

134   CHAR Tregs were co-cultured with irradiated PA2.1 or IVA12 B-cell hybridoma cell lines at a 1:1

135   ratio in RPMI10 medium supplemented with 1,000 IU/ml IL-2 in round-bottom 96-wells. Tregs

136   alone served as a negative control. Cells were passaged and expanded and expression of the

137   Treg lineage transcription factors FOXP3 and HELIOS was assessed 9 days later by intracellular

138   staining using the FOXP3/Transcription Factor Staining Buffer Set (eBioscience, ThermoFisher

139   Scientific), according to manufacturer's instructions. Teff cells were stained for FOXP3 and

140   HELIOS as negative controls.

141

142   ***Cytotoxicity assay***

143   CHAR Tregs, CHAR Teff cells or their untransduced (UT) counterparts were co-incubated with

144   PA2.1 cells at a 1:1 ratio for 24 hours in round-bottom 96-wells. 50 μl supernatant was then

145   carefully removed and target cell killing assessed using the CyQUANT Cytotoxicity Lactate

146   Dehydrogenase (LDH) Release Assay kit (Thermofisher Scientific) as per manufacturer's

147   instructions [33].

148

149   ***Allogeneic B cell stimulation***

150   Peripheral blood mononuclear cells (PBMCs) from de-identified HLA-A2 pre-sensitized patients

151   (Paediatric and Adult Nephrology Unit, La Paz University Hospital, Madrid, Spain) were thawed

152   in RPMI10, counted, and the frequency of B cells (CD19$^+$CD20$^+$ cells) was determined by spectral

153   flow cytometry. On the same day, PBMCs were incubated with 100 IU/ml IL-2, 100 IU /ml IL-6

154 (Peprotech, ThermoFisher Scientific) [34], and irradiated (4,000 rad) HLA2-expressing K562 cells

155 (a kind gift from Jack Strominger, Harvard University) at a ratio of 1 B cell : 10 irradiated HLA-A2-

156 K562 cells with or without HLA-A2 CHAR Tregs at a ratio of 1 CHAR Treg : 1 B cell in 12-wells

157 for up to 5 days. PBMCs from pre-sensitized patients with IL-2 and IL-6 alone, as well as PBMCs

158 from a de-identified healthy donor (STEMCELL Technologies) subjected to all 3 conditions (Figure

159 3B), were kept as negative controls.

160

161 ***IgG antibody production assay***

162 Three conditions were set up with HLA-A2 pre-sensitized patient PBMCs and healthy donor

163 PBMCs: PBMCs alone (with IL-2 and IL-6), PBMCs with irradiated HLA2-K562 cells, and PBMCs

164 with irradiated HLA2-K562 cells and HLA-A2 CHAR Tregs, as described above (Figure 3B). 250

165 μl supernatant was collected from each allogeneic B cell stimulation condition at 48h and 5 days

166 and diluted 1:2 with PBS. Human IgG solid-phase sandwich enzyme-linked immunosorbent assay

167 (ELISA) (Thermofisher Scientific) was performed as per manufacturer's instructions.

168

169 ***Spectral flow cytometry***

170 Spectral flow cytometry data was acquired in a 3-laser Cytek Northern Lights spectral flow

171 cytometer (Cytek Biosciences, Fremont, CA). Spectroflow 3.2.1 (Cytek Biosciences) and FlowJo

172 v10.9 software (BD Life Sciences, Franklin Lakes, NJ) were used for flow cytometry data analysis.

173 Data were manually pre-gated to remove cell aggregates, dead cells, debris, and then sub-

174 sampled to include 10,000 live singlets from each sample. Uniform Manifold Approximation and

175 Projection (UMAP) analysis was performed to visualize the different sub-populations in groups

176 [35]. UMAP settings were as follows: all files used, all compensated fluorescent parameters were

177 used besides Live/Dead,Neighbors =15, Minimum Distance = 0.5, Components = 2, Metric =

178 Euclidean. Antibodies used for spectral flow cytometry in this study can be found in

179 Supplementary Table 1.

180

181 **Results**

182 Aiming to test the concept of regulatory T cell (Tregs) engineered to specifically recognize and

183 inhibit alloreactive B cells responsible for human leukocyte antigen (HLA) pre-sensitization in

184 patients, we constructed a chimeric anti-HLA antibody receptor and UT (CHAR) specific for anti-

185 HLA-A2 B cells by fusing an HLA-A2 molecule to a CD28 hinge, a CD28 transmembrane domain,

186 and a CD28-CD3zeta intracellular signaling domain [27; 28] (Figure 1A). We then sorted

187 $CD4^+CD25^{hi}CD127^{low}$ Tregs [30; 31] from human peripheral blood collected from HLA-A2

188 negative donors, activated them with anti-CD3/CD28 beads and IL-2, and transduced them two

189 days later with lentivirus coding for the HLA-A2 CHAR. Flow cytometry analysis of transduced

190 Tregs revealed successful expression of the CHAR construct, as assessed by simultaneous

191 surface expression of HLA-A2 and the truncated nerve growth factor receptor (NGFRt) reporter

192 gene, linked to CHAR gene expression via a 2A peptide (Figure 1B).

193

194 To evaluate CHAR target recognition, we co-incubated CHAR Tregs with B-cell hybridoma cell

195 lines specific for HLA-A2 (PA2.1) or for HLA-DR (IVA12). CHAR Tregs upregulated surface

196 expression of the T cell activation marker CD69 (Figure 1C) and increased in frequency (Figure

197 1D) upon co-incubation with PA2.1 cells, but not with IVA12 cells, indicating CHAR Treg reactivity

198 specifically to HLA-A2 antibody-producing cells.

199

200 Next, we sought to confirm that activation via the CHAR did not compromise Treg identity stability,

201 measured by the expression level of the Treg lineage transcription factors FOXP3 and HELIOS

202 (REFs). We found no difference in the frequency of FOXP3 or HELIOS expressing cells between

203 untransduced (UT) or CHAR Tregs co-incubated with either PA2.1 or IVA12 hybridoma cells

204 (Figure 2A). Of note, while the frequency of $FOXP3^+$ CHAR Tregs (Figure 2B) and

205 $FOXP3^+HELIOS^+$ CHAR Tregs (Figure 2D) did not change across conditions, FOXP3 levels were

206   higher in CHAR Tregs co-incubated with PA2.1 cells (Figure 2C, an additional line of evidence for

207   CHAR Treg activation specifically by anti-HLA-A2 antibody-producing cells.

208

209   Interestingly, CHAR Tregs were not cytotoxic towards anti-HLA-A2 antibody-producing PA2.1

210   cells at a 1:1 ratio, unlike CHAR T effector (Teff) cells (Figure 2E), further confirming that HLA-A2

211   CHAR activation does not destabilize Treg identity.

212

213   To assess CHAR Treg function, we thawed peripheral blood mononuclear cells (PBMCs) from

214   HLA-A2 pre-sensitized patients (SEN) and a healthy donor (HD) (Figure 3A) and co-incubated

215   them with irradiated HLA-A2-expressing K562 cells as a source of HLA-A2 antigen in the

216   presence of IL-2 and IL-6 with or without CHAR Tregs for 2 days or 5 days (Figure 3B). In two out

217   of three HLA-A2 pre-sensitized patients tested, CHAR Tregs significantly decreased IgG antibody

218   production by the patient's cells after 48h of co-incubation (Figure 6), demonstrating the ability of

219   CHAR Tregs to inhibit alloreactive B cells.

220

221   In order to gain mechanistic insight into CHAR Treg function, we performed spectral flow

222   cytometric analysis of these co-cultures. We identified NGFR+HLA-A2+CD19- CHAR Tregs and

223   NGFR-HLA-A2-CD19+CD20+ patient B cells, which we then subsetted into naive (CD27$^-$IgD$^+$),

224   marginal zone (CD27$^+$IgD$^+$), memory (CD27$^+$IgD$^-$), and CD27$^-$IgD$^-$ B cells (Figure 4A). Uniform

225   Manifold Approximation and Projection (UMAP) visualization of total live cells in the different co-

226   culture conditions across all three pre-sensitized patients at 48 hours (Figure 4B) and 5 days

227   (Figure 4D) illustrates that B cells constitute a small and relatively uniform fraction of the patients'

228   PBMCs (colored in blue, light green, dark green, and orange according to B cell subset) and that

229   CHAR Tregs (colored in red) form distinct clusters, potentially reflecting differences in activation

230   status. Focusing on the B cell fraction, we found that CHAR Tregs significantly reduced the

231   frequency of total B cells in all three pre-sensitized patients' PBMCs at 48 hours (Figure 4B) and

232   at 5 days (Figure 4D) of co-culture. Of note, all B-cell subsets measured (naïve, memory, marginal

233   zone, and CD27⁻IgD⁻ B cells) were equally depleted by HLA-A2 CHAR Tregs at both time points

234   (Figure 4B, 4D). With regards to the CHAR Tregs, we observed that the majority of CHAR Tregs

235   expressed high levels of CD27, a marker associated with Treg suppressive function [36] after 48h

236   (Figure 4C) and 5 days (Figure 4E) of co-culture. There was a trend where a larger fraction of

237   CHAR Tregs expressed CD27 when co-incubated with SEN PBMCs (86% at 48 hours, 76-88%

238   at 5 days) than with HD PBMCs (80% at 48 hours, 68% at 5 days) (Figure 4C, 4E). Moreover,

239   CHAR Tregs were activated, as assessed by CD71 upregulation [29], in the presence of all three

240   SEN PBMCs, but not HD PBMCs after 48 hours (Figure 4C) and 5 days (Figure 4E) of co-culture.

Discussion

242   The field of immunotherapy has witnessed remarkable advancements in recent years, particularly

243   concerning the engineering of T cells to target specific cells and immune responses. Previous

244   studies have demonstrated the potential of conventional T cells to recognize and eliminate B cells.

245   In the clinic, total B cells are being eliminated using CD19 chimeric antigen receptor (CAR) T cells

246   in patients with systemic lupus erythematosus and other autoimmune disorders, leading to

247   disease remission [37; 38]. At the pre-clinical stage, self-reactive antigen-specific B cells have

248   been eliminated through chimeric autoantibody receptor T cells (CAAR T cells) in the setting of

249   pemphigus vulgaris [39] and alloreactive HLA-specific B cells have been targeted using chimeric

250   HLA receptor (CHAR) T cells [27; 28].

251   Our study introduces a novel approach by engineering regulatory T cells (Tregs), immune cells

252   dedicated to inhibiting immune responses to maintain immune homeostasis [21; 22; 23], to

253   recognize and suppress B cells specific for an HLA molecule with a CHAR. This innovative

254   strategy not only enhances the specificity of Treg-mediated suppression of alloreactive B cells

255   but also opens new avenues for therapeutic intervention in transplant immunology. Tregs, like all

256   CD4^{+} T cells, possess T cell receptors (TCRs) restricted to human leukocyte antigen (HLA) class

257   II molecules, which are expressed in professional antigen presenting cells, including B cells [40].

258   Previous work reported the expansion of rare recipient alloreactive Tregs using donor-derived B

259   cells [41]. Yet, antigen-specific Tregs are rare and prone to destabilization upon multiple rounds

260   of activation *ex vivo*, kindling interest in genetic engineering approaches to confer desired antigen

261   specificities to human Tregs [21; 22]. Previous work has focused on modifying human Tregs with

262   artificial receptors to confer them specificity towards transplanted tissues, aiming to provide

263   localized protection from immune attack [42; 43; 44; 45; 46]. Engineering Tregs with CHAR

264   tackles the problem of allogeneic immune rejection from a different, potentially orthogonal and

265   synergistic, angle, providing a targeted mechanism to directly inhibit B cell responses against

266   allogeneic HLA molecules, such as HLA-A2. Unlike engineered conventional T cells, which can

267   induce wide-ranging immune activation and potential tissue damage [47; 48; 49; 50], HLA-A2

268   CHAR Tregs are designed to suppress only B cells producing anti-HLA-A2 antibodies in an anti-

269   inflammatory fashion, thereby minimizing collateral damage to other components of the immune

270   system or any tissues. In line with this concept, we observed that conventional HLA-A2 CHAR T

271   cells were cytotoxic towards anti-HLA-A2 antibody-producing hybridoma cells, whereas HLA-A2

272   CHAR Tregs were not (Figure 2E). This specificity and safety profile is crucial in the context of

273   transplantation, where current desensitization strategies often fall short due to their non-specific

274   nature and associated complications [19]. By effectively reducing IgG antibody production in

275   sensitized individuals (Figure 3B), CHAR Tregs offer a promising therapeutic option for improving

276   transplant outcomes by enhancing graft acceptance and reducing the risk of rejection.

277   Engineering of Tregs to bind specific B cells had been previously demonstrated with FVIII B cell

278   antibody receptor (BAR) Tregs, aimed at inhibiting anti-FVIII antibody production by hemophilic

279   patients treated with recombinant FVIII protein [51]. While this work demonstrated the possibility

280   of directing Tregs towards B cells based on B-cell antigen specificity, our findings extend the

281 applicability of B-cell-targeting engineered Tregs to graft-vs-host disease, organ transplantation,

282 and beyond, potentially addressing challenges in conditions such as miscarriage, where pregnant

283 women develop antibodies against paternally derived HLA molecules [52].

284 The novelty of our study lies not only in the engineering of Tregs with CHAR but also in the

285 demonstration of their efficacy with HLA sensitized patients' cells. The ability of CHAR Tregs to

286 suppress IgG production by B cells from pre-sensitized individuals upon exposure to antigen-

287 expressing cells (Figure 3B) is a significant step forward, suggesting that this approach could

288 pave the way for tailored strategies that address individual HLA sensitized patient needs.

289 Polyclonal Tregs have been shown to be safe in phase I and phase II clinical trials [53; 54; 55],

290 and human CAR Tregs targeting transplanted tissues have shown efficacy and safety in

291 preclinical studies [44; 56; 57; 58] and are being tested in ongoing phase I clinical trials [59].

292 Hence, CHAR Tregs are a good candidate for first-in-human trials, to be used either in pre-

293 sensitized patients with the goal of bringing them to the same baseline as non-sensitized patient

294 and/or as an adjuvant to reduce the doses of immunosuppressive drugs taken by transplant

295 recipients.

296 Interestingly, CHAR Tregs significantly inhibited IgG production by HLA-A2 antigen-stimulated B

297 cells for only two out of three sensitized patients (Figure 3B). Moreover, CHAR Tregs retained

298 high expression of the activation marker CD71 after 5 days of co-culture also only with two out of

299 three sensitized patient PBMCs (Figure 4E). Future studies are warranted to define what patient

300 characteristics indicate responsiveness to CHAR Treg targeting, as well as to expand the concept

301 to other HLA class I and class II molecules and dissect the mechanisms by which CHAR Tregs

302 inhibit target B cells.

303 In conclusion, we provide compelling proof-of-concept for a novel immunotherapeutic strategy to

304 desensitize transplant recipients with HLA sensitization. Engineering CHAR Tregs represents an

305 advancement in the quest for specific and safe immunotherapies to modulate harmful B-cell

306 responses in sensitized transplant recipients and beyond. As we continue to refine these

307 technologies, the potential for engineered Tregs to transform clinical practice becomes a

308 tantalizing prospect.

309

310 **Data availability statement**

311 The raw data supporting the conclusions of this article will be made available by the authors,

312 without undue reservation.

313

314 **Author contributions**

315 JVQ designed the project, designed experiments, conducted experiments, analyzed data, and

316 wrote the manuscript. AZS provided samples and analyzed data. ELC analyzed data. LMRF

317 designed the project, supervised work, designed experiments, conducted experiments, analyzed

318 data, and wrote the manuscript. The manuscript was edited and approved by all authors.

319

320 **Funding**

329

Figure legends

Figure 1. HLA-A2 CHAR Tregs are activated specifically by anti-HLA-A2 antibody producing cells. (A) Schematic representation of chimeric anti-human leukocyte antigen (HLA) antibody receptor (CHAR) featuring a CD28-CD3 zeta signaling domain, expressed on the surface of a human regulatory T cell (Treg) binding an anti-HLA-A2 antibody on the surface of an allogeneic B cell from an HLA-A2 pre-sensitized patient. The B cell receptor (BCR) on the surface of a B cell comprises a surface-bound antibody and the signaling heterodimer CD79A and CD79B. Upon engagement, the HLA-A2 CHAR Treg suppresses anti-HLA-A2 expressing B cell function. **(B)** Cell surface expression of HLA-A2 CHAR construct in lentivirus transduced Tregs, as assessed by co-expression of HLA-A2 and a reporter gene, truncated nerve growth factor receptor (NGFRt), linked to the CHAR gene by a 2A peptide. UT, untransduced. **(C)** HLA-A2

355   CHAR Treg activation, as assessed by surface expression of CD69, upon 48-hour co-incubation

356   with irradiated PA2.1 (anti-HLA-A2) and IVA12 (anti-HLA-DR, DP, DQ) hybridoma cells. **(D)**

357   Enrichment of HLA-A CHAR expressing Tregs upon 9-day co-incubation with irradiated PA2.1

358   and IVA12 hybridoma cells, as assessed by surface expression of NGFRt reporter. For **(C)** and

359   **(D)**, bars represent mean and standard deviation (n = 3 technical replicates). Data were analyzed

360   by one-way ANOVA with multiple comparisons. ns, not significant; *p < 0.05, **p < 0.01, ***p <

361   0.001, and ****p < 0.0001.

362   **Figure 2. HLA-A2 CHAR Tregs remain stable and are not cytotoxic upon activation. (A)**

363   Representative flow cytometry analysis of CHAR Treg FOXP3 and HELIOS expression after 9

364   days of co-culture with irradiated PA2.1 (anti-HLA-A2) and IVA12 (anti-HLA-DR, DP, DQ)

365   hybridoma cells. Untransduced (UT) T effector (Teff) cells and CHAR Teff cells were used as

366   negative controls. **(B)** Frequency of FOXP3$^+$ CHAR Tregs alone or co-cultured with PA2.1 and

367   IVA12 hybridoma cells for 9 days. **(C)** FOXP3 expression (mean fluorescence intensity - MFI) in

368   CHAR Tregs alone or co-cultured with PA2.1 and IVA12 hybridoma cells for 9 days. **(D)**

369   Frequency of FOXP3+HELIOS+ CHAR Tregs alone or co-cultured with irradiated PA2.1 and

370   IVA12 hybridoma cells for 9 days. **(E)** Cytotoxicity of HLA-A2 CHAR Tregs and HLA-A2 CHAR

371   Teff cells towards PA2.1, as measured by lactate dehydrogenase (LDH) release after 24-hour co-

372   incubation at a 1:1 ratio**.** Bars in (B), (C), (D), and (E) represent mean and standard deviation (n

373   = 3 technical replicates). Data were analyzed by one-way ANOVA with multiple comparisons. ns,

374   not significant; *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

375   **Figure 3.HLA-A2 CHAR Tregs suppress IgG specific production on highly pre sensitized**

376   **patients. (A)** Experimental design for assessing HLA-A2 CHAR Treg function in the presence of

377   HLA-A2 pre-sensitized patient cells. HLA-A2 sensitized donor-derived peripheral blood

378   mononuclear cells (PBMCs) were co-incubated with HLA-A2-expressing K562 cells to induce

379   expansion of anti-HLA-A2 B cells and anti-HLA-A2 IgG antibody production. If HLA-A2 CHAR

380 Tregs are added, a decrease in antibody production elicited by exposure to HLA-A2 is expected.

381 **(B)** IgG antibody production 48 hours or 5 days after pre-sensitized patient PBMC co-incubation

382 with HLA-A2-K562 in the presence or absence of HLA-A2 CHAR T regs, as assessed by ELISA

383 (n=3 sensitized patients (SEN) and n=1 healthy donor (HD) control). Data were analyzed by one-

384 way ANOVA with multiple comparisons. ns, not significant; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$,

385 and $****p < 0.0001$. **(C)** Patient and healthy control demographics and clinical characteristics.

386 **Figure 4.HLA-A2 CHAR Tregs were activated and reduced the frequency of total B cells in**

387 **pre-sensitized patient peripheral blood samples. (A)** Flow cytometry gating strategy to identify

388 HLA-A2$^+$NGFR$^+$CD19$^-$ CHAR Tregs, CD19$^+$CD20$^+$ B cells, and B cell subsets in co-cultures of

389 sensitized patient PBMCs with irradiated HLA-A2-K562 and HLA-A2 CHAR Tregs. **(B,D)** Uniform

390 manifold approximation and projection (UMAP) representation of sensitized patient PBMCs with

391 irradiated HLA-A2-K562 (iA2K562) and HLA-A2 CHAR Tregs depicting naive, marginal zone,

392 memory and IgD$^-$CD27$^-$ B cells after 48 hours **(B)** and 5 days **(D)** of co-culture. Red subset

393 represents HLA-A2 CHAR Treg in all UMAPs. **(C,E)** Frequency of CD27 expression (left) and

394 CD71 surface expression levels (right) in HLA-A2 CHAR Tregs co-incubated with sensitized

395 patient PBMCs for 48 hours **(C)** and 5 days **(E)**. n=3 sensitized patients (SEN) and n=1 healthy

396 donor (HD) control. Data were analyzed by one-way ANOVA with multiple comparisons. ns, not

397 significant; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

398

399 **References**
400
401 [1] R. Boenink, A. Kramer, R.E. Tuinhout, E. Savoye, A. Asberg, A. Idrizi, J. Kerschbaum, I.
402 Ziedina, E. Ziginskiene, E. Farrugia, L. Garneata, E.V. Zakharova, S. Bell, M. Arnol, M.
403 Segelmark, K. Ioannou, K. Hommel, M. Rosenberg-Ots, E. Vazelov, J. Helve, S. Mihaly,
404 R. Palsson, M. Nordio, N. Gjorgjievski, A.P.J. de Vries, N. Seyahi, W.A. Magadi, H. Resic,
405 A. Kalachyk, A.O. Rahmel, A.A. Galvao, R. Naumovic, T. Lundgren, M. Arici, J.M. de
406 Meester, A. Ortiz, K.J. Jager, and V.S. Stel, Trends in kidney transplantation rate across
407 Europe: study from the ERA Registry. Nephrol Dial Transplant 38 (2023) 1528-1539.
408 [2] Global Observatory on Donation and Transplantation, International Report on Organ Donation
409 and Transplantation Activities, 2022.

410 [3] Q. Li, and P. Lan, Activation of immune signals during organ transplantation. Signal Transduct
411   Target Ther 8 (2023) 110.
412 [4] E. Au, G. Wong, and J.R. Chapman, Cancer in kidney transplant recipients. Nat Rev Nephrol
413   14 (2018) 508-520.
414 [5] R. Ruiz, and A.D. Kirk, Long-Term Toxicity of Immunosuppressive Therapy. in: R.W. Busuttil,
415   and G.B.G. Klintmalm, (Eds.), Transplantation of the Liver, Elsevier, 2015, pp. 1354-1363.
416 [6] R. Ettenger, H. Chin, K. Kesler, N. Bridges, P. Grimm, E.F. Reed, M. Sarwal, R. Sibley, E.
417   Tsai, B. Warshaw, and A.D. Kirk, Relationship Among Viremia/Viral Infection,
418   Alloimmunity, and Nutritional Parameters in the First Year After Pediatric Kidney
419   Transplantation. Am J Transplant 17 (2017) 1549-1562.
420 [7] J. Nelson, N. Alvey, L. Bowman, J. Schulte, M.C. Segovia, J. McDermott, H.S. Te, N. Kapila,
421   D.J. Levine, R.L. Gottlieb, J. Oberholzer, and M. Campara, Consensus recommendations
422   for use of maintenance immunosuppression in solid organ transplantation: Endorsed by
423   the American College of Clinical Pharmacy, American Society of Transplantation, and the
424   International Society for Heart and Lung Transplantation. Pharmacotherapy 42 (2022)
425   599-633.
426 [8] V. Shivaswamy, B. Boerner, and J. Larsen, Post-Transplant Diabetes Mellitus: Causes,
427   Treatment, and Impact on Outcomes. Endocr Rev 37 (2016) 37-61.
428 [9] A. Tsampalieros, G.A. Knoll, A.O. Molnar, N. Fergusson, and D.A. Fergusson, Corticosteroid
429   Use and Growth After Pediatric Solid Organ Transplantation: A Systematic Review and
430   Meta-Analysis. Transplantation 101 (2017) 694-703.
431 [10] M.L. Stuber, Psychiatric issues in pediatric organ transplantation. Pediatr Clin North Am 58
432   (2011) 887-901, x.
433 [11] A. Francis, D.W. Johnson, J.C. Craig, and G. Wong, Incidence and Predictors of Cancer
434   Following Kidney Transplantation in Childhood. Am J Transplant 17 (2017) 2650-2658.
435 [12] K.E. Order, and N.M. Rodig, Pediatric Kidney Transplantation: Cancer and Cancer Risk.
436   Semin Nephrol 44 (2024) 151501.
437 [13] J. Hyun, K.D. Park, Y. Yoo, B. Lee, B.Y. Han, E.Y. Song, and M.H. Park, Effects of different
438   sensitization events on HLA alloimmunization in solid organ transplantation patients.
439   Transplant Proc 44 (2012) 222-5.
440 [14] M.M. Colvin, J.L. Cook, P.P. Chang, D.T. Hsu, M.S. Kiernan, J.A. Kobashigawa, J.
441   Lindenfeld, S.C. Masri, D.V. Miller, E.R. Rodriguez, D.B. Tyan, A. Zeevi, F. American
442   Heart Association Heart, C. Transplantation Committee of the Council on Clinical, Y.
443   Council on Cardiovascular Disease in the, C. Council on, N. Stroke, S. Council on
444   Cardiovascular, and Anesthesia, Sensitization in Heart Transplantation: Emerging
445   Knowledge: A Scientific Statement From the American Heart Association. Circulation 139
446   (2019) e553-e578.
447 [15] N.M. Valenzuela, and E.F. Reed, Antibodies in transplantation: the effects of HLA and non-
448   HLA antibody binding and mechanisms of injury. Methods Mol Biol 1034 (2013) 41-70.
449 [16] D. Middleton, P. Hamilton, C.C. Doherty, J.F. Douglas, and M.G. McGeown, Mismatching for
450   HLA-A, -B antigens and renal graft survival. Clin Nephrol 23 (1985) 26-7.
451 [17] S. Barocci, U. Valente, and A. Nocera, Detection and analysis of HLA class I and class II
452   specific alloantibodies in the sera of dialysis recipients waiting for a renal retransplantation.
453   Clin Transplant 21 (2007) 47-56.
454 [18] D.A. Axelrod, M.A. Schnitzler, T. Alhamad, F. Gordon, R.D. Bloom, G.P. Hess, H. Xiao, M.
455   Nazzal, D.L. Segev, V.R. Dharnidharka, A.S. Naik, N.N. Lam, R. Ouseph, B.L. Kasiske,
456   C.M. Durand, and K.L. Lentine, The impact of direct-acting antiviral agents on liver and
457   kidney transplant costs and outcomes. Am J Transplant 18 (2018) 2473-2482.
458 [19] R. Leal, C. Pardinhas, A. Martinho, H.O. Sa, A. Figueiredo, and R. Alves, Strategies to
459   Overcome HLA Sensitization and Improve Access to Retransplantation after Kidney Graft
460   Loss. J Clin Med 11 (2022).

461 [20] O. Aubert, M.C. Bories, C. Suberbielle, R. Snanoudj, D. Anglicheau, M. Rabant, F. Martinez,
462   A. Scemla, C. Legendre, and R. Sberro-Soussan, Risk of antibody-mediated rejection in
463   kidney transplant recipients with anti-HLA-C donor-specific antibodies. Am J Transplant
464   14 (2014) 1439-45.
465 [21] L.M.R. Ferreira, Y.D. Muller, J.A. Bluestone, and Q. Tang, Next-generation regulatory T cell
466   therapy. Nat Rev Drug Discov 18 (2019) 749-769.
467 [22] F. Ghobadinezhad, N. Ebrahimi, F. Mozaffari, N. Moradi, S. Beiranvand, M. Pournazari, F.
468   Rezaei-Tazangi, R. Khorram, M. Afshinpour, R.A. Robino, A.R. Aref, and L.M.R. Ferreira,
469   The emerging role of regulatory cell-based therapy in autoimmune disease. Front Immunol
470   13 (2022) 1075813.
471 [23] S. Sakaguchi, T. Yamaguchi, T. Nomura, and M. Ono, Regulatory T cells and immune
472   tolerance. Cell 133 (2008) 775-87.
473 [24] B. Sawitzki, P.N. Harden, P. Reinke, A. Moreau, J.A. Hutchinson, D.S. Game, Q. Tang, E.C.
474   Guinan, M. Battaglia, W.J. Burlingham, I.S.D. Roberts, M. Streitz, R. Josien, C.A. Boger,
475   C. Scotta, J.F. Markmann, J.L. Hester, K. Juerchott, C. Braudeau, B. James, L. Contreras-
476   Ruiz, J.B. van der Net, T. Bergler, R. Caldara, W. Petchey, M. Edinger, N. Dupas, M.
477   Kapinsky, I. Mutzbauer, N.M. Otto, R. Ollinger, M.P. Hernandez-Fuentes, F. Issa, N.
478   Ahrens, C. Meyenberg, S. Karitzky, U. Kunzendorf, S.J. Knechtle, J. Grinyo, P.J. Morris,
479   L. Brent, A. Bushell, L.A. Turka, J.A. Bluestone, R.I. Lechler, H.J. Schlitt, M.C. Cuturi, S.
480   Schlickeiser, P.J. Friend, T. Miloud, A. Scheffold, A. Secchi, K. Crisalli, S.M. Kang, R.
481   Hilton, B. Banas, G. Blancho, H.D. Volk, G. Lombardi, K.J. Wood, and E.K. Geissler,
482   Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design
483   and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet 395 (2020)
484   1627-1639.
485 [25] Q. Tang, J. Leung, Y. Peng, A. Sanchez-Fueyo, J.J. Lozano, A. Lam, K. Lee, J.R. Greenland,
486   M. Hellerstein, M. Fitch, K.W. Li, J.H. Esensten, A.L. Putnam, A. Lares, V. Nguyen, W.
487   Liu, N.D. Bridges, J. Odim, A.J. Demetris, J. Levitsky, T. Taner, and S. Feng, Selective
488   decrease of donor-reactive T(regs) after liver transplantation limits T(reg) therapy for
489   promoting allograft tolerance in humans. Sci Transl Med 14 (2022) eabo2628.
490 [26] A. Cassano, A.S. Chong, and M.L. Alegre, Tregs in transplantation tolerance: role and
491   therapeutic potential. Front Transplant 2 (2023) 1217065.
492 [27] S. Betriu, J. Rovira, C. Arana, A. Garcia-Busquets, M. Matilla-Martinez, M.J. Ramirez-Bajo,
493   E. Banon-Maneus, M. Lazo-Rodriguez, A. Bartolo-Ibars, F.H.J. Claas, A. Mulder, S. Heidt,
494   M. Juan, B. Bayes-Genis, J.M. Campistol, E. Palou, and F. Diekmann, Chimeric HLA
495   antibody receptor T cells for targeted therapy of antibody-mediated rejection in
496   transplantation. HLA 102 (2023) 449-463.
497 [28] I. Gille, R.S. Hagedoorn, E.M.W. van der Meer-Prins, M.H.M. Heemskerk, and S. Heidt,
498   Chimeric HLA antibody receptor T cells to target HLA-specific B cells in solid organ
499   transplantation. HLA 102 (2023) 436-448.
500 [29] R.W. Cochrane, R.A. Robino, B. Granger, E. Allen, S. Vaena, M.J. Romeo, A.A. de Cubas,
501   S. Berto, and L.M.R. Ferreira, High-affinity chimeric antigen receptor signaling induces an
502   inflammatory program in human regulatory T cells. Mol Ther Methods Clin Dev 32 (2024)
503   101385.
504 [30] W. Liu, A.L. Putnam, Z. Xu-Yu, G.L. Szot, M.R. Lee, S. Zhu, P.A. Gottlieb, P. Kapranov, T.R.
505   Gingeras, B. Fazekas de St Groth, C. Clayberger, D.M. Soper, S.F. Ziegler, and J.A.
506   Bluestone, CD127 expression inversely correlates with FoxP3 and suppressive function
507   of human CD4+ T reg cells. J Exp Med 203 (2006) 1701-11.
508 [31] N. Seddiki, B. Santner-Nanan, J. Martinson, J. Zaunders, S. Sasson, A. Landay, M. Solomon,
509   W. Selby, S.I. Alexander, R. Nanan, A. Kelleher, and B. Fazekas de St Groth, Expression
510   of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and
511   activated T cells. J Exp Med 203 (2006) 1693-700.

[32] R.W. Cochrane, R.A. Robino, and L.M.R. Ferreira, Generation of Human Chimeric Antigen Receptor Regulatory T Cells. J Vis Exp (2025).

[33] C.M. Zimmerman, R.A. Robino, R.W. Cochrane, M.D. Dominguez, and L.M.R. Ferreira, Redirecting Human Conventional and Regulatory T Cells Using Chimeric Antigen Receptors. Methods Mol Biol 2748 (2024) 201-241.

[34] K. Maeda, H. Mehta, D.A. Drevets, and K.M. Coggeshall, IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood 115 (2010) 4699-706.

[35] J.M. Benito, D. Jimenez-Carretero, C. Restrepo, J.M. Ligos, J. Valentin-Quiroga, I. Mahillo, A. Cabello, E. Lopez-Collazo, F. Sanchez-Cabo, M. Gorgolas, V. Estrada, N. Rallon, and E.I.i.t.S.A.R. Network, T Cell Homeostasis Disturbances in a Cohort of Long-Term Elite Controllers of HIV Infection. Int J Mol Sci 25 (2024).

[36] R.C. Duggleby, T.N. Shaw, L.B. Jarvis, G. Kaur, and J.S. Gaston, CD27 expression discriminates between regulatory and non-regulatory cells after expansion of human peripheral blood CD4+ CD25+ cells. Immunology 121 (2007) 129-39.

[37] A. Mackensen, F. Muller, D. Mougiakakos, S. Boltz, A. Wilhelm, M. Aigner, S. Volkl, D. Simon, A. Kleyer, L. Munoz, S. Kretschmann, S. Kharboutli, R. Gary, H. Reimann, W. Rosler, S. Uderhardt, H. Bang, M. Herrmann, A.B. Ekici, C. Buettner, K.M. Habenicht, T.H. Winkler, G. Kronke, and G. Schett, Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med 28 (2022) 2124-2132.

[38] X. Wang, X. Wu, B. Tan, L. Zhu, Y. Zhang, L. Lin, Y. Xiao, A. Sun, X. Wan, S. Liu, Y. Liu, N. Ta, H. Zhang, J. Song, T. Li, L. Zhou, J. Yin, L. Ye, H. Lu, J. Hong, H. Cheng, P. Wang, W. Li, J. Chen, J. Zhang, J. Luo, M. Huang, L. Guo, X. Pan, Y. Jin, W. Ye, L. Dai, J. Zhu, L. Sun, B. Zheng, D. Li, Y. He, M. Liu, H. Wu, B. Du, and H. Xu, Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. Cell 187 (2024) 4890-4904 e9.

[39] C.T. Ellebrecht, V.G. Bhoj, A. Nace, E.J. Choi, X. Mao, M.J. Cho, G. Di Zenzo, A. Lanzavecchia, J.T. Seykora, G. Cotsarelis, M.C. Milone, and A.S. Payne, Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science 353 (2016) 179-84.

[40] T. Kinnunen, N. Chamberlain, H. Morbach, J. Choi, S. Kim, J. Craft, L. Mayer, C. Cancrini, L. Passerini, R. Bacchetta, H.D. Ochs, T.R. Torgerson, and E. Meffre, Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. Blood 121 (2013) 1595-603.

[41] L.M. Lee, H. Zhang, K. Lee, H. Liang, A. Merleev, F. Vincenti, E. Maverakis, A.W. Thomson, and Q. Tang, A Comparison of Ex Vivo Expanded Human Regulatory T Cells Using Allogeneic Stimulated B Cells or Monocyte-Derived Dendritic Cells. Front Immunol 12 (2021) 679675.

[42] K.G. MacDonald, R.E. Hoeppli, Q. Huang, J. Gillies, D.S. Luciani, P.C. Orban, R. Broady, and M.K. Levings, Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. J Clin Invest 126 (2016) 1413-24.

[43] Y.D. Muller, L.M.R. Ferreira, E. Ronin, P. Ho, V. Nguyen, G. Faleo, Y. Zhou, K. Lee, K.K. Leung, N. Skartsis, A.M. Kaul, A. Mulder, F.H.J. Claas, J.A. Wells, J.A. Bluestone, and Q. Tang, Precision Engineering of an Anti-HLA-A2 Chimeric Antigen Receptor in Regulatory T Cells for Transplant Immune Tolerance. Front Immunol 12 (2021) 686439.

[44] J.M. Barra, R.A. Robino, R. Castro-Gutierrez, J. Proia, H.A. Russ, and L.M.R. Ferreira, Combinatorial genetic engineering strategy for immune protection of stem cell-derived beta cells by chimeric antigen receptor regulatory T cells. Cell Rep 43 (2024) 114994.

[45] W. Du, F. Noyan, O. McCallion, V. Drosdek, J. Kath, V. Glaser, C. Fuster-Garcia, M. Yang, M. Stein, C. Franke, Y. Pu, O. Weber, J.K. Polansky, T. Cathomen, E. Jaeckel, J. Hester, F. Issa, H.D. Volk, M. Schmueck-Henneresse, P. Reinke, and D.L. Wagner, Gene editing

563   of CD3 epsilon to redirect regulatory T cells for adoptive T cell transfer. Mol Ther 33 (2025)
564   997-1013.
565 [46] R.W. Cochrane, E. Allen, and L.M.R. Ferreira, Expanding the engineered Treg multiverse.
566   Mol Ther 33 (2025) 833-836.
567 [47] C.H. June, and M. Sadelain, Chimeric Antigen Receptor Therapy. N Engl J Med 379 (2018)
568   64-73.
569 [48] K.M. Cappell, and J.N. Kochenderfer, Long-term outcomes following CAR T cell therapy:
570   what we know so far. Nat Rev Clin Oncol 20 (2023) 359-371.
571 [49] A.R. Haas, R.J. Golden, L.A. Litzky, B. Engels, L. Zhao, F. Xu, J.A. Taraszka, M. Ramones,
572   B. Granda, W.J. Chang, J. Jadlowsky, K.M. Shea, A. Runkle, A. Chew, E. Dowd, V.
573   Gonzalez, F. Chen, X. Liu, C. Fang, S. Jiang, M.M. Davis, N.C. Sheppard, Y. Zhao, J.A.
574   Fraietta, S.F. Lacey, G. Plesa, J.J. Melenhorst, K. Mansfield, J.L. Brogdon, R.M. Young,
575   S.M. Albelda, C.H. June, and J.L. Tanyi, Two cases of severe pulmonary toxicity from
576   highly active mesothelin-directed CAR T cells. Mol Ther 31 (2023) 2309-2325.
577 [50] L.M.R. Ferreira, and Y.D. Muller, CAR T-Cell Therapy: Is CD28-CAR Heterodimerization Its
578   Achilles' Heel? Front Immunol 12 (2021) 766220.
579 [51] A.H. Zhang, J. Yoon, Y.C. Kim, and D.W. Scott, Targeting Antigen-Specific B Cells Using
580   Antigen-Expressing Transduced Regulatory T Cells. J Immunol 201 (2018) 1434-1441.
581 [52] H.S. Nielsen, M.D. Witvliet, R. Steffensen, G.W. Haasnoot, E. Goulmy, O.B. Christiansen,
582   and F. Claas, The presence of HLA-antibodies in recurrent miscarriage patients is
583   associated with a reduced chance of a live birth. J Reprod Immunol 87 (2010) 67-73.
584 [53] J.A. Bluestone, J.H. Buckner, M. Fitch, S.E. Gitelman, S. Gupta, M.K. Hellerstein, K.C.
585   Herold, A. Lares, M.R. Lee, K. Li, W. Liu, S.A. Long, L.M. Masiello, V. Nguyen, A.L.
586   Putnam, M. Rieck, P.H. Sayre, and Q. Tang, Type 1 diabetes immunotherapy using
587   polyclonal regulatory T cells. Sci Transl Med 7 (2015) 315ra189.
588 [54] C. Bender, A.E. Wiedeman, A. Hu, A. Ylescupidez, W.K. Sietsema, K.C. Herold, K.J. Griffin,
589   S.E. Gitelman, S.A. Long, T.R.S. Groupdagger, and T.R.C.S. Group, A phase 2
590   randomized trial with autologous polyclonal expanded regulatory T cells in children with
591   new-onset type 1 diabetes. Sci Transl Med 16 (2024) eadn2404.
592 [55] E.H. Meyer, G. Laport, B.J. Xie, K. MacDonald, K. Heydari, B. Sahaf, S.W. Tang, J. Baker,
593   R. Armstrong, K. Tate, C. Tadisco, S. Arai, L. Johnston, R. Lowsky, L. Muffly, A.R.
594   Rezvani, J. Shizuru, W.K. Weng, K. Sheehan, D. Miklos, and R.S. Negrin, Transplantation
595   of donor grafts with defined ratio of conventional and regulatory T cells in HLA-matched
596   recipients. JCI Insight 4 (2019).
597 [56] D.A. Boardman, C. Philippeos, G.O. Fruhwirth, M.A. Ibrahim, R.F. Hannen, D. Cooper, F.M.
598   Marelli-Berg, F.M. Watt, R.I. Lechler, J. Maher, L.A. Smyth, and G. Lombardi, Expression
599   of a Chimeric Antigen Receptor Specific for Donor HLA Class I Enhances the Potency of
600   Human Regulatory T Cells in Preventing Human Skin Transplant Rejection. Am J
601   Transplant 17 (2017) 931-943.
602 [57] F. Noyan, K. Zimmermann, M. Hardtke-Wolenski, A. Knoefel, E. Schulde, R. Geffers, M. Hust,
603   J. Huehn, M. Galla, M. Morgan, A. Jokuszies, M.P. Manns, and E. Jaeckel, Prevention of
604   Allograft Rejection by Use of Regulatory T Cells With an MHC-Specific Chimeric Antigen
605   Receptor. Am J Transplant 17 (2017) 917-930.
606 [58] L.M. Ferreira, and Q. Tang, Generating Antigen-Specific Regulatory T Cells in the Fast Lane.
607   Am J Transplant 17 (2017) 851-853.
608 [59] K. Schreeb, E. Culme-Seymour, E. Ridha, C. Dumont, G. Atkinson, B. Hsu, and P. Reinke,
609   Study Design: Human Leukocyte Antigen Class I Molecule A(*)02-Chimeric Antigen
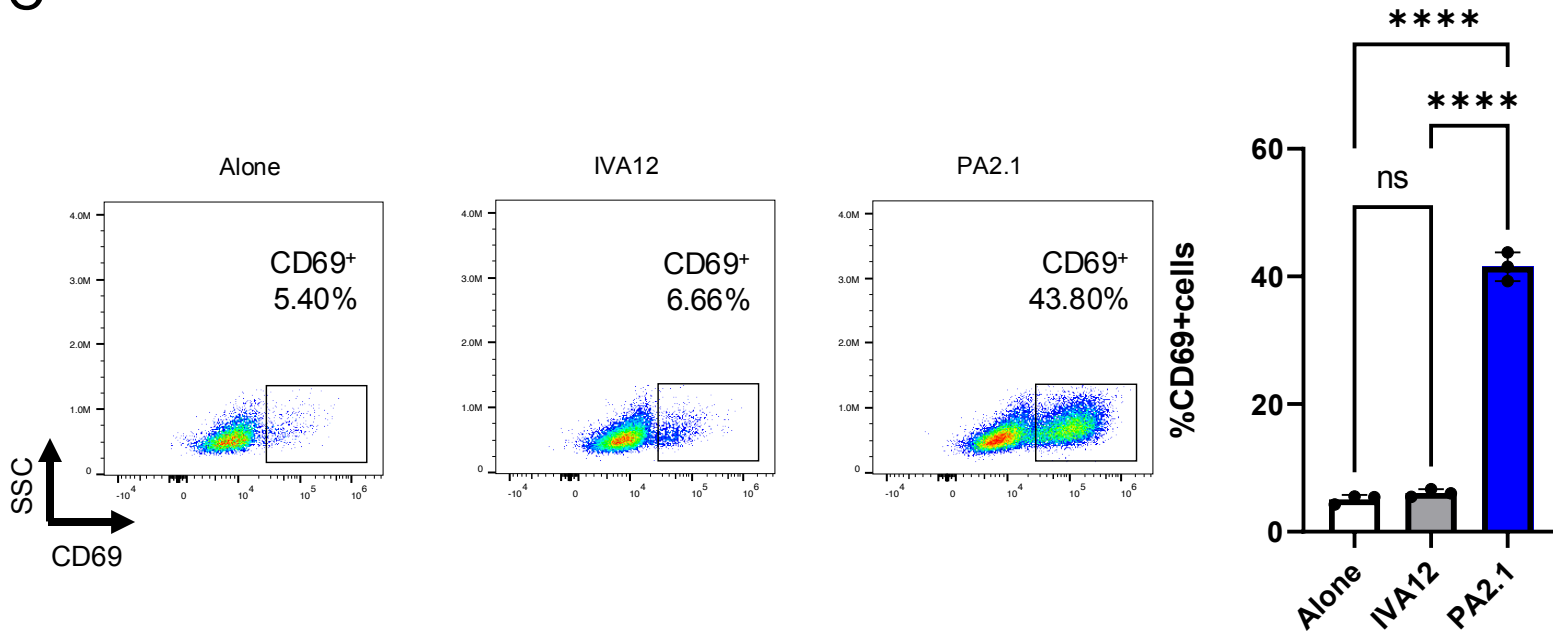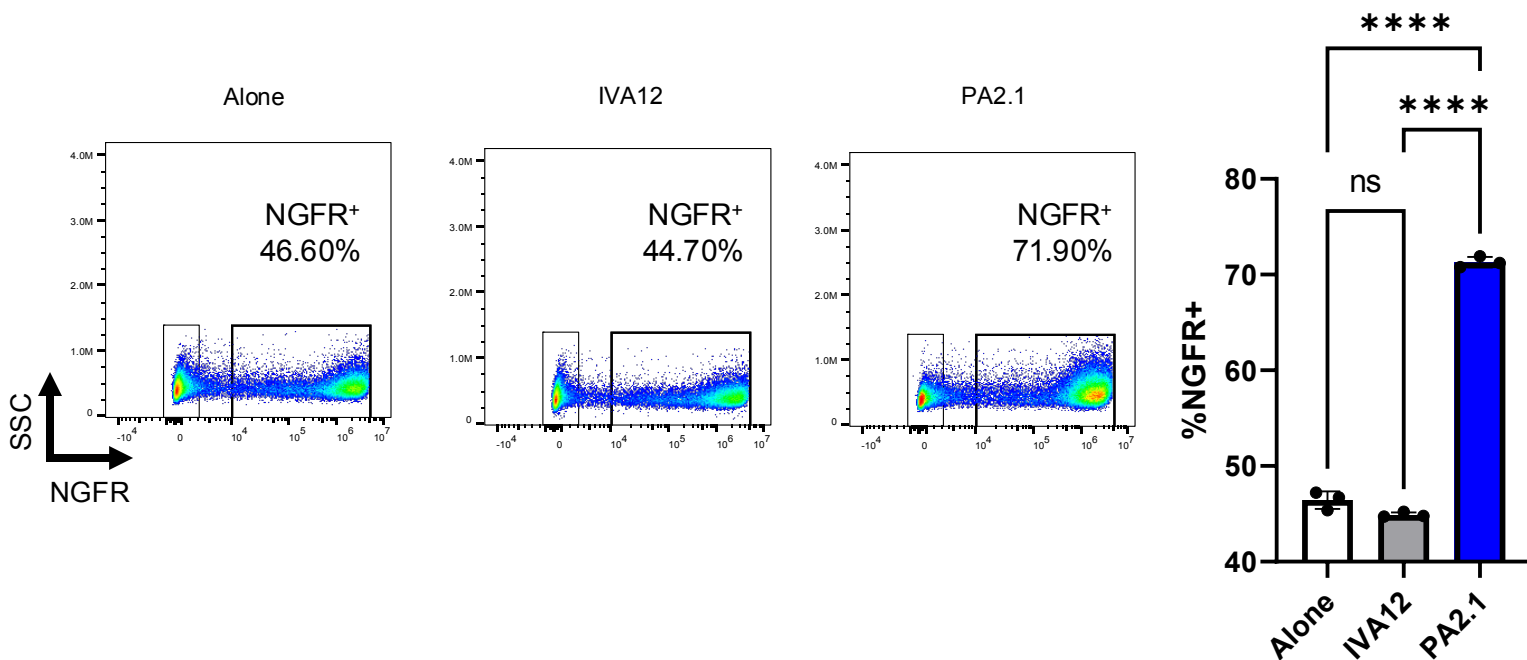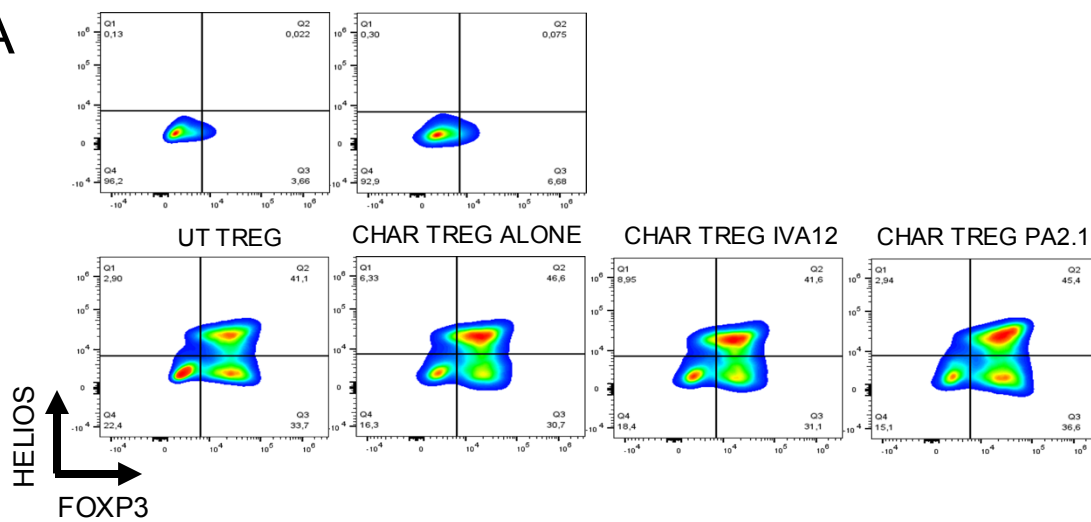610   Receptor Regulatory T Cells in Renal Transplantation. Kidney Int Rep 7 (2022) 1258-
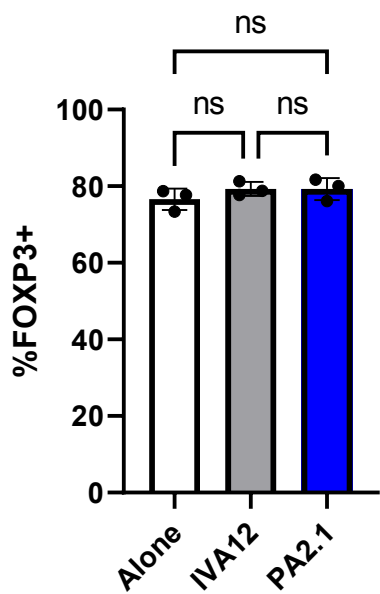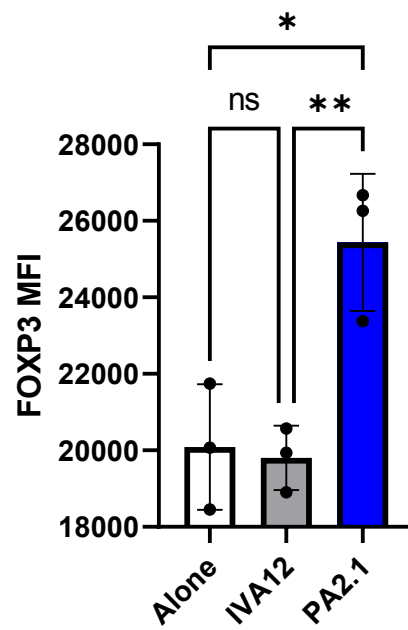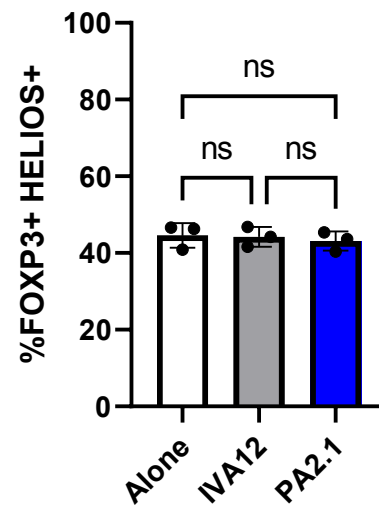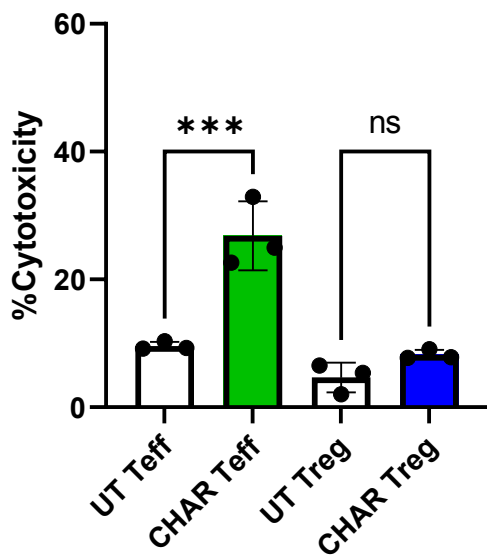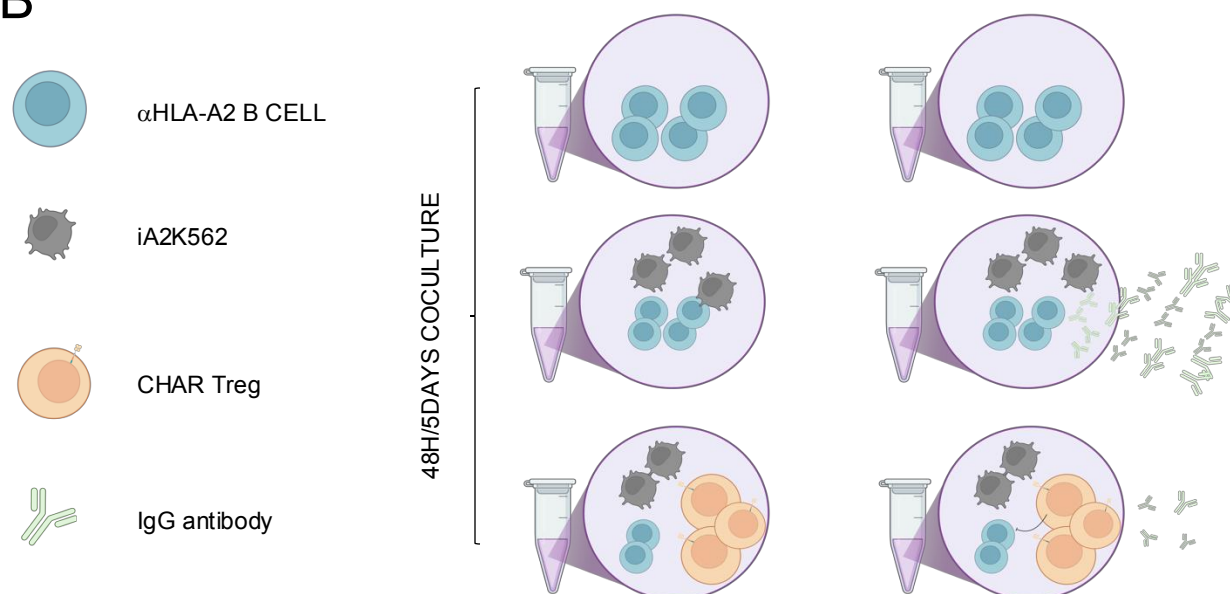611   1267.
612

A
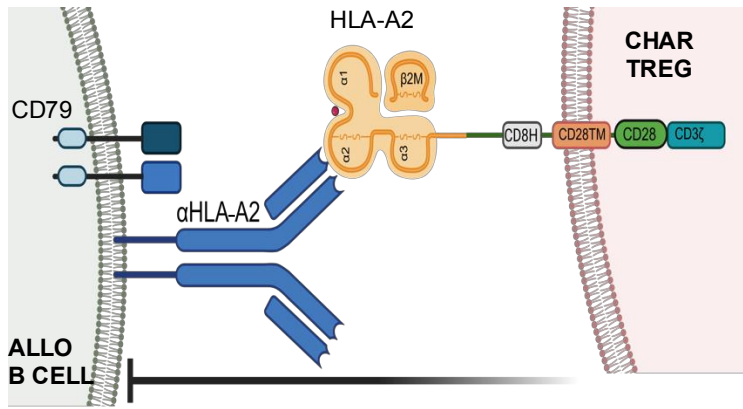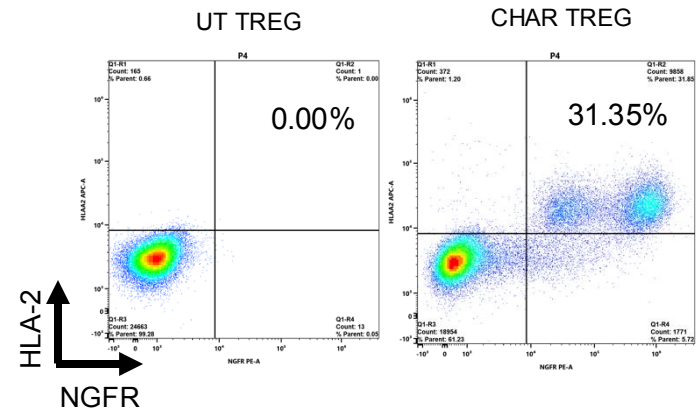
SAMPLE	SEX	AGE	HEMODIALYSIS (YEARS)	NUMBER OF TRANSPLANTS	IMMUNOSUPRESSION REGIMEN	HLA TYPING	αHLA CLASS I (MFI)	αHLA-A2 (MFI)
HEALTHY DONOR	FEMALE	26	NA	NA	NA	A*01, A*01 / B*08, B*51 / DRB1*01, DRB1*03	NA	NA
SENSITIZED1	MALE	11	6	2	TACROLIMUS MYCOPHENOLATE THYMOGLOBULIN (6 TIMES) RITUXIMAB (2 TIMES) BASILIXIMAB	A*09, A*33 / B*14, B*44 / DRB1*01, DRB1*07	HIGH MFI>300000	MODERATE MFI>2000
SENSITIZED2	MALE	46	6	NA	TACROLIMUS	A*03, A*26 / B*18, B*35 / DRB1*01, DRB1*03	MODERATE MFI>100000	HIGH MFI>4000
SENSITIZED3	FEMALE	17	1	2	TACROLIMUS MYCOPHENOLATE BASILIXIMAB	A*24, A*66 / B*41, B*44 / DRB1*07, DRB1*13	HIGH MFI>230000	HIGH MFI>4000

B

C